Using ObjectJ for fish oocyte and egg measurements; Hough transform, particle analysis, manual size measurements, and categorization

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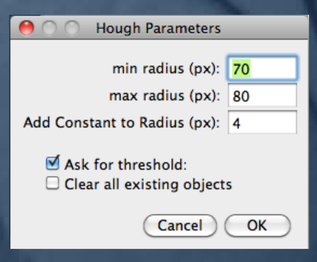
INTRODUCTION

In egg surveys and reproductive studies on fish it is often necessary to work up egg and oocyte samples. Samples of live pelagic eggs usually consist of perfectly round and highly transparent eggs that need to have their diameter measured and categorized based on species and/or stage. Sometimes these egg samples are fixed in formalin before they are analyzed, then the eggs become more opaque, but with transparent parts. Oocyte samples from maturing fish mostly consist of opaque, vitellogenic oocytes that can be more or less round or oval. Also the oocytes need to be diameter measured and categorized.

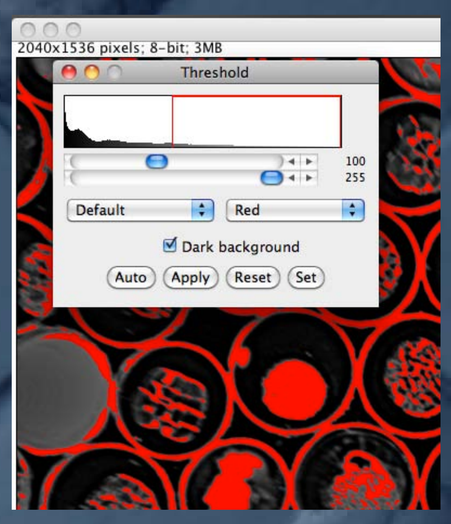
To help us in analyzing these three sample types we used ImageJ with the plug-in ObjectJ (http://simon.bio.uva.nl/objectj). ObjectJ is a plug-in for ImageJ that supports graphical vector objects that non-destructively mark images on a transparent layer. Composite objects can encapsulate different color-coded marker structures in order to bundle features that belong together. The results table supports statistics, sorting, color coding, qualifying and macro access.

Hough transform; diameter measurements and categorization of live transparent eggs

For the automatic diameter measurements the user can chose to measure one image at a time or operate in batch mode for analysis of many pictures. The typical speed of the automatic diameter measurements is 150-250 eggs/minute.

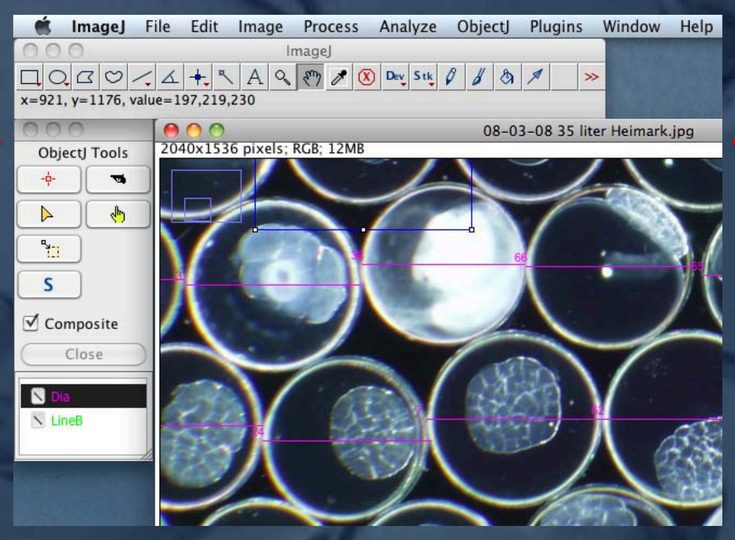


Settings for Hough transform: Minimum and maximum possible egg radius, constant adjustment factor, and threshold adjustment option

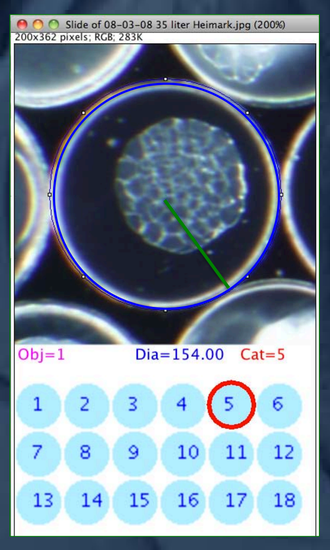


Thresholding is performed automatically or manually

Hough transform is performed on skeletonized picture

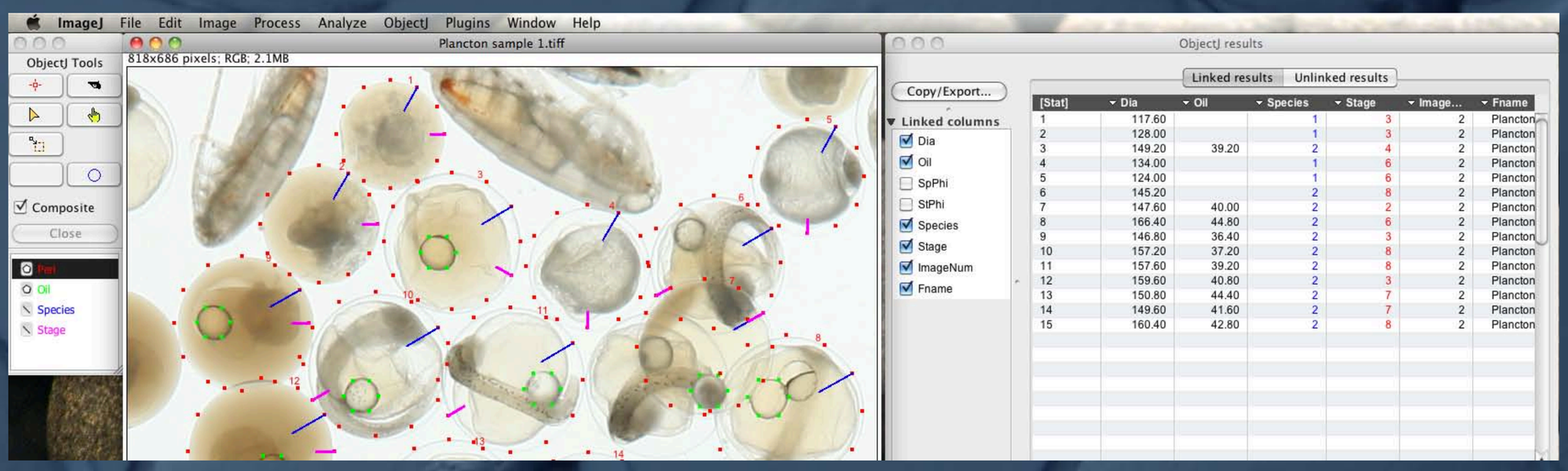


Result of Hough transform. Egg diameters are marked with horizontal lines



Slide show: The automatically measured diameter can be adjusted and the egg can be assigned a category. Category is displayed using a hand of a clock analogy (green line).

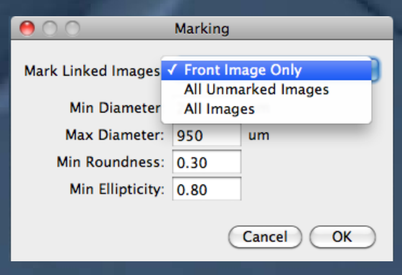
Manual diameter analysis and categorization of partly transparent fixed mackerel eggs



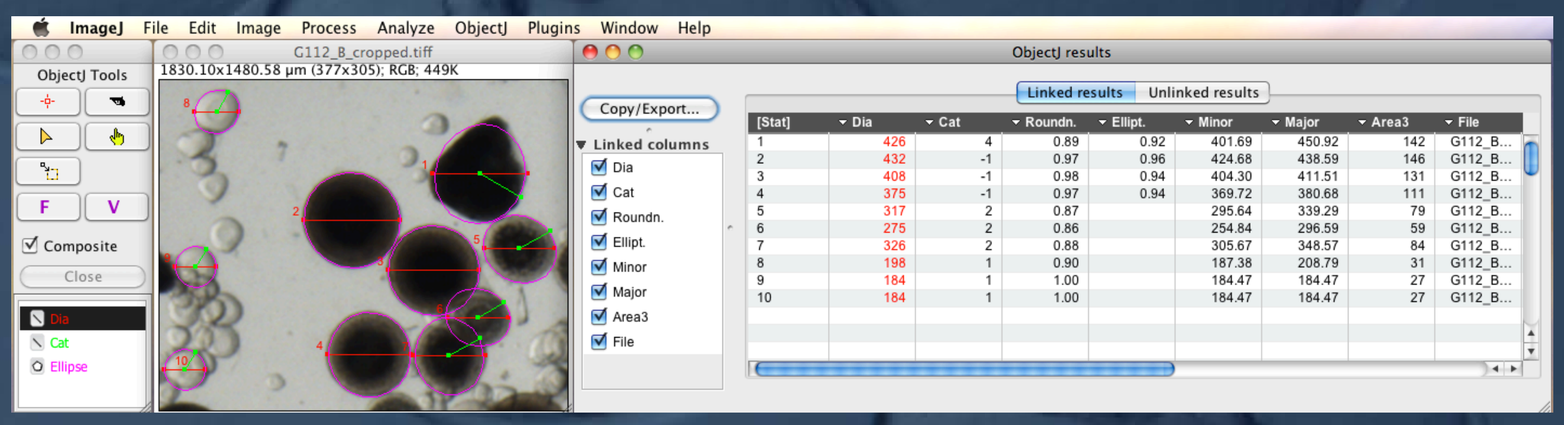
Red dots mark the perimeter of the egg (diameter) and green dots marks the perimeter of the lipid droplet. Hands of a clock analogy displays egg stage (pink) and species (blue).

Results window shows results for each egg and overall statistics. Results are hot-linked to image display.

Automatic particle analysis of opaque fish oocytes



Images can be analyzed one by one or in batch mode. Threshold values can be set for diameter, roundness, and ellipticity.



Each measured oocyte is marked with the best fitting ellipse and the corresponding diameter recalculated for a perfect sphere with the same silhouette area. Hand of a clock analogy is used to display manually assigned categories (green lines).

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